IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Matthew B. Wheeler

Serial No.: 08/410,539

Filed: March 24, 1995

For: TRANSGENIC UNGULATE METHODS AND COMPOSITIONS

Group Art Unit: 1819

Examiner: Bruce Campbell

MAIN BRIEF ON APPEAL TO THE BOARD OF PATENT APPEALS AND INTERFERENCES

1. Real Party In Interest

The real parties in interest in this Application are co-assignees Biotechnology Research and Development Corp. and the Board of Trustees of the University of Illinois.

2. Related Appeals And Interferences

None.

3. Status Of Claims

This Application originally contained claims 1-77, claims 78-79 were added by Preliminary Amendment, and claims 14, 22-77 were canceled as not elected in this Application, prior to calculating the filing fee for this Application. Claims 1-13, 15-21, 78 and 79 remained pending in this Application. In an amendment filed concurrently with the present Appeal Brief, claims 7-8, 13, 21 and 78-79 are canceled. Claims 1-6, 9-12 and 15-20 are the object of this Appeal. A copy of the pending claims appears as an Appendix to this Brief.

Claims have been issued in the parent Wheeler U.S. Patent 5,523,226, on June 4, 1996. Claims not elected in the present application have been allowed in Wheeler U.S. Ser. No. 08/473,030. Other claims not elected in this Application or in '030 remain as potential for continuing applications.

4. Status Of Amendments

An amendment is filed concurrently with the present Appeal Brief after the final rejection of January 9, 1998. A Supplemental Information Disclosure Statement was filed September 28, 1998.

5. Summary Of The Invention[1]

According to an aspect of the invention claimed in this application as Claims 1-6 and 9-12, a novel and successful method for making a chimeric ungulate uses ungulate embryonic stem (ES) cells in culture (page 11, lines 1-6), cells that have a first genetic complement (page 11, lines 18-21). The recipient of the ES cells is an embryo of the same species (page 11, line 21)that has a second genetic complement (page 11, lines 33-34). The first and the second genetic complement may differ in the proteins they encode, which allows useful products from other species such as human proteins to be produced by ungulates (page 11, lines 13-17; page 12, lines 32-35 to page 13, lines 1-7).

Another aspect of the invention appears in Claims 15-20, a method of isolating and purifying an ungulate embryonic stem culture (page 13, lines 20-35 to page 14, line 22). The inventors are the first to successfully produce totipotent ES cell types from ungulates and to produce chimeric ungulates (pages 51-56).

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[1] Cites are to the specification.

6. Issues Presented

A. Rejections Applied

Claims 1-6, 9-12 and 15-20 were rejected under 35 U.S.C. § 112, first paragraph, because the specification is not enabling for all ungulates.

B. Main Issues Raised by Rejections.

Whether examples from two genera, swine and sheep, are adequate to enable the family "ungulates."

7. Grouping Of Claims

Claims 1-6, 9-12 are separately patentable, and do not stand or fall with claims 15-20. This is because claims to a method of making a chimeric ungulate (1-6, 9-12) use the ungulate ES cells of claims 15-20, but are an independent achievement. The ungulate ES cells of claims 15-20 have other, independent uses, e.g. to produce proteins in cultures.

8. Argument

The Specification is Enabling for Ungulates

Claims 1-6, 9-12 and 15-20 were rejected under 35 U.S.C. § 112, first paragraph.

The Examiner's first basis for this rejection is:

> The specification does not provide any working examples or specific guidance regarding production of embryonic stem (ES) cells of species other than swine . . . .

(Action of March 17, 1997, page 3.) The Examiner provided no justification under the law that a "working example" must be present for enablement. Nor did the Examiner indicate what he would require as a "working example." In the Office Actin of January 9, 1998, the Examiner admitted that there is no legal requirement for a working example. The Examiner argues this is just "one of the factors to be considered when determining whether a

specification is enabling," (Action of January 9, 1998, page 3), but provides no other factors. A similar lack of support renders the following bald conclusion by the Examiner unable to legally support a rejection:

> Hence, those skilled in the art would not accept the ES-like cells described in the declaration as "true" ES cells based solely on their appearance, particularly since true sheep ES cells have not been produced previously.

Action of January 9, 1998, page 4.

Therefore, the Examiner's basis for the enablement rejections are not legally sufficient.

With regard to "specific guidance," that the Examiner says is lacking in the specification (Action of January 9, 1998, page 3), guidance is presented on page 22 of the application about how to select suitable ungulate embryos from which ES cells are derived. On pages 23-26 of the specification, detailed steps are presented for initiating and maintaining ungulate embryo cultures, isolating ungulate ES cells, and maintaining ES cells in culture. The morphological features of ES cells and cultures are found on pages 14-15 of the specification.

Tips for isolating ES cells from pigs and sheep are presented on page 24, lines 11-25. Table 3 describes ES cell morphology of ungulates. Porcine and sheep ES colonies are described on pages 24-25. Determining the modal chromosome member to see if an ES culture is stable, is described on page 26 for pigs, cattle, sheep and goats. Guidance on how to maintain ES cells in culture is presented on pages 26-28 for pigs and sheep. Table 2, page 44, presents comparative methods of making ES cell lines in pigs, cattle, sheep and goats.

Methods for producing chimeric ungulates from ES cells are given on pages 29-31, including methods to introduce genes by manipulation of the ES cells, and methods for identifying chimeras (pages 31-32).

Suitable first and second breeds of swine, goats, sheep and cattle and suitable markers for these species are presented on page 32. (*See also* Materials and Methods, in particular the "Collection of Swine, Bovine, Ovine and Caprine Embryos and Isolation of ES-Like Cells," pp. 69-75.)

In addition, the Examiner doubts the assertion in the application that the claimed methods work for ungulates and other sheep, despite a declaration from the inventor, Dr. Wheeler, that attests that sheep ES cells were prepared following the protocols of the present specification (Exhibit A).

Although it is true that there are some differences in embryonic development among ungulates, these differences are not shown by the Examiner to affect response of the different ungulate species to the methods of the present invention for producing chimeras.

With regard to the Examiner's reliance on the Piedrahita et al. (A52) publication, only potential swine stem cells were isolated. Piedrahita was unable to maintain "ES" cell lines or to demonstrate the isolated cells' pluripotency. Therefore, there is no evidence ES cells were produced. There was no assertion that ovine embryos produced ES cells. The methods disclosed by Piedrahita could not be described as "a method to produce embryonic stem cells," which implies substantial homogeneity and reproducibility, neither of which were demonstrated. Applicant maintains that ES cells were not produced by Piedrahita et al.,

therefore, the Examiner's concern "that porcine and ovine embryos responded differently to the same treatments" (Action, page 3) is not supported by Piedrahita.

The Examiner points to "differences" among species reported by Cruz et al. and by Bazer et al., but selectively fails to mention similarities among ungulates described in these publications, e g., "The pattern of development during cleavage is similar for all farm species studied" (*see* Table 8-2). Table 8-2 relates cattle, horse, sheep and swine. Therefore, it is reasonable to expect the invention is suitable for all ungulates.

The need for undue experimentation is not a necessary consequence of differences among species. The same protocols described in detail in the specification, and admitted by the Examiner to be "enabling for swine" (Action of March 17, 1997, page 3), may be applied to other ungulates.

Methods in the art to "validate" ES cells are in the specification as, e.g. on page 10, as acknowledged in the Action of March 17, 1997, page 3. Therefore, those practicing the invention can readily determine whether or not they have produced ES cells by following the guidelines of the invention. Production of tumors in an immunocompromised mammal is another method of verification.

The Examiner mentions Applicant's comments about species differences. Concerns in the application are about extrapolating from mice to ungulates (application, page 5, lines 4-8) that is, concerns about differences in embryonic development among widely diverse biological (taxonomic) categories. Extrapolating from swine to other ungulates is extrapolating with a family, therefore development should be more similar. Indeed, similarities among ungulates and differences between ungulates and rodents are discussed in

the application on page 8, lines 26-35; page 9, lines 1-35; page 10, lines 1-5; and page 22, lines 8-26.

Because ungulate embryological development is similar among the member species, and ES cells and chimeric pigs have been produced from pigs and, according to the application and Dr. Wheeler's declaration, ES cells have been produced from sheep, the production of chimeric ungulates other than swine should not require undue experimentation.

9. Conclusion

The Examiner errs in rejecting claims 1-6, 9-12 and 15-20 under 35 U.S.C. § 112, first paragraph because he maintains that two ungulate genera examples - swine and sheep are insufficient to support claims to all ungulates.

The Examiner errs in rejecting claims 1-6, 9-12 and 15-20 because he doubts Dr. Wheeler's declaration relating sheep ES cells because it does not provide evidence they are:

> "incorporated into all cell types of an organism, particularly the germ line."

Accordingly, the Board is requested to REVERSE the Final Rejection of January 9, 1998.

Respectfully submitted,

November 9, 1998

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Appendix: Claims on Appeal

1. A method for making a chimeric ungulate comprising:

(a) introducing an ungulate embryonic stem cell that has a first genetic complement into a recipient embryo of the same species as the embryonic stem cell, said recipient having a second genetic complement, to form a chimeric ungulate embryo; and

(b) placing the chimeric ungulate embryo in an environment suitable for the completion of development to form a chimeric ungulate.

2. The method of claim 1, wherein the ungulate embryonic stem cell is pluripotent.

3. The method of claim 2, wherein the ungulate embryonic stem cell is totipotent.

4. The method of claim 1, wherein the embryonic stem cell is introduced into the embryo at a pre-implantation stage.

5. The method of claim 4, wherein the pre-implantation stage is the blastocyst stage.

6. The method of claim 1, wherein the embryonic stem cell is derived from a first breed of ungulate and the recipient embryo is derived from a second breed of the same species as the first breed.

9. The method of claim 1, wherein the first genetic complement is different from the second genetic complement.

10. The method of claim 9, wherein the first genetic complement comprises an exogenous nucleotide sequence stably integrated into the genetic complement of the embryonic stem cell.

11. The method of claim 10, wherein the first genetic complement comprises a nucleotide sequence capable of being expressed to provide human Factor IX in recoverable form from the chimeric ungulate.

12. The method of claim 10, wherein the first genetic complement comprises a nucleotide sequence encoding a protein selected from the group consisting of human blood proteins, human hormones, human growth factors, human cytokines, human enzymes, human hormone receptors, human binding proteins, antigens, translation factors, transcription factors, onco-proteins, protooncoproteins, human milk proteins, and human muscle proteins.

15. A method of isolating and purifying an embryonic stem cell culture, said method comprising:

(a) preparing a first culture by culturing dissociated cells from an ungulate embryo in conditioned stem cell medium in the absence of a feeder layer; and

(b) subculturing the first culture until a second stable culture with morphological features and growth parameters characteristic of an embryonic stem cell culture is established.

16. The method of claim 15, wherein the dissociated cells from an ungulate embryo are obtained from an ungulate embryo which was developed *in vitro* in stem cell medium (SCM) on a feeder layer.

17. The method of claim 15, wherein the stem cell medium is conditioned by Buffalo Rat Liver Cells, and includes growth factors, vitamins, amino acids and antibiotics.

18. The method of claim 17, wherein the stem cell conditioned medium (CSCM) comprises approximately 40% of stem cell medium (SCM) and approximately 60% of Buffalo Rat Liver Cell conditioned medium (BRL/CM).

19. The method of claim 15, wherein the morphological features of cells isolated from the culture comprise a round shape, as observed with the light microscope, a diameter of approximately 8-15 microns, and a cytoplasmic to nuclear diameter ratio of approximately 10-25:75-90, and wherein the growth parameters of the cells in culture comprise a doubling time of approximately 18-36 hours and multilayered rather than monolayered growth.

20. The method of claim 15, further defined as producing an embryonic stem cell culture which comprises at least 50% of cells that are capable of forming a teratoma or a teratocarcinoma when introduced into a host mouse.